PB86-156296

# Microbial Destructions Achieved by Full-Scale Anaerobic Digestion

(U.S.) Environmental Protection Agency
Cincinnati, OH

Jan 86

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| 1. REPORT NO. | 2. | 3. R PB86-156296 |
|---|---|---|
| EPA/600/D-86/031 | | |

| 4. TITLE AND SUBTITLE | 5. REPORT DATE |
|---|---|
| MICROBIAL DESTRUCTIONS ACHIEVED BY FULL-SCALE ANAEROBIC DIGESTION | January 1986 |
| | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) | 8. PERFORMING ORGANIZATION REPORT NO. |
| J.B. Farrell, G. Stern, and A.D. Venosa | |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS | 10. PROGRAM ELEMENT NO. |
| Water Engineering Research Laboratory<br>Office of Research and Development<br>U.S. Environmental Protection Agency<br>Cincinnati, OH 45268 | 11. CONTRACT/GRANT NO.<br>Inhouse |
| 12. SPONSORING AGENCY NAME AND ADDRESS | 13. TYPE OF REPORT AND PERIOD COVERED |
| Water Engineering Research Laboratory-Cincinnati, OH<br>Office of Research and Development<br>U.S. Environmental Protection Agency<br>Cincinnati, OH 45268 | Proceedings Paper<br>14. SPONSORING AGENCY CODE<br>EPA/600/14 |

15. SUPPLEMENTARY NOTES
Contact = Dr. J.B. Farrell, 513-569-7645

16. ABSTRACT

As part of studies undertaken to investigate the pathogen reducing capabilities of conventional sludge stabilization procedures, microbial reductions produced by mesophilic and thermophilic digestion at the Los Angeles Hyperion Plant were examined. Samples from the digester feed and product streams were collected over a 2-year period and sent to EPA's Cincinnati Laboratory for bacterial, viral, and parasite determination. These results were compared with results from two Ohio treatment plants.

From the Los Angeles sludge data, reductions in certain bacteria and viruses by mesophilic anaerobic digestion can be defined--as can defensible confidence limits. These data could not be supplemented with information on bacterial reductions produced by other digesters because the densities of Salmonella sp. in the influent of these other digesters were not sufficiently high. The unusual result of a multistage digester showing bacterial reductions equivalent to a single stage digester, under equivalent residence time, should be further investigated. Although the data were preliminary, reductions in Ascaris sp. by thermophilic digestion at 49°C were poor.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| | | |

| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS *(This Report)* | 21. NO. OF PAGES |
|---|---|---|
| RELEASE TO PUBLIC | UNCLASSIFIED | 20 |
| | 20. SECURITY CLASS *(This page)* | 22. PRICE |
| | UNCLASSIFIED | |

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION REPRODUCED BY

NATIONAL TECHNICAL
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EPA/600/D-86/031
January 1986

# MICROBIAL DESTRUCTIONS ACHIEVED BY FULL-SCALE ANAEROBIC DIGESTION

by

J. B. FARRELL, G. STERN, and A. D. VENOSA

## INTRODUCTION

In the early 1970's, it became evident to staff in the Wastewater Research Division of EPA's Municipal Environmental Research Laboratory (now Water Engineering Research Laboratory-WERL) in Cincinnati that regulations would eventually be established restricting the use of sludge because of its potential for transmitting disease. Since the degree of restriction that might be created could not be predicted, a program was instituted in which the pathogen reducing capabilities of conventional sludge stabilization procedures were investigated. Also attention was given to developing and evaluating stabilization and/or treatment procedures which produce higher reductions in the density of pathogenic organisms in sludge than conventional processes. Processes investigated included conventional and thermophilic aerobic digestion, lime stabilization, long-term lagooning, as well as mesophilic and thermophilic anaerobic digestion. The program was carried out by in-house research, grants, and contracts, and by cooperative research with other investigators. Much of this work has appeared in the literature.[1-5]

One particularly successful program was conducted with the staff of the City of Los Angeles Hyperion Plant, in which microbiological reductions produced by mesophilic and thermophilic digestion was documented. Cooperators were Messrs. Garber, Ohara and Nagano of the City of Los Angeles. At this point, mesophilic digestion had been successfully practiced for many years and at the time of this program, one of the several digesters was operated in the thermophilic mode. Samples drawn from the mesophilic and thermophilic digester feed and product streams were collected over a two-year period and sent to EPA's Laboratory in Cincinnati for bacterial, viral, and parasite determination. The information collected proved invaluable in providing a data base for supporting EPA's regulations for pathogen reduction required before sludge use on the land.[6] Unfortunately, results of this study have only been fragmentarily reported.[7-10]

Further work on documenting the bacterial reductions achieved at other sewage treatment plants (Fairfield and Lebanon, Ohio) was carried out by the Cincinnati Laboratory relatively recently and compared with the Los Angeles results. This comparison brought to light some unique features and findings of the Los Angeles work that had never been properly presented. Examination of reviews on the subject of microbial reductions in sludge treatment processes[11-13] showed that no publications of plant-scale results

were as extensive or as complete. Consequently, this paper has been prepared to present the entire Los Angeles study and compare its results with the more recent results obtained with the Ohio treatment plants.

The objective of the Los Angeles and the Ohio studies was to determine pathogen reductions achieved by anaerobic digestion under normal conditions of operation at full-scale municipal wastewater treatment plants. The Los Angeles study was more complete--fate of viruses, parasites, Salmonella sp., and bacterial indicators were determined. At the Ohio plants, only fate of Salmonella sp. and bacterial indicators was determined.

## EXPERIMENTAL PROCEDURES

### Los Angeles Experiments

The Los Angeles Hyperion plant provides primary treatment to 1.3 M cu m/day of wastewater; of this amount 0.4 M $m^3$/day receives secondary treatment by the activated sludge process. All sludge is digested. The digesters and their operation at Los Angeles have been well-described by Garber.[14] Raw sludge, which is a mixture of primary and waste activated solids, is pumped continuously to the digesters; three of the 12 (or more) digesters receive feed at any given time. Each digester is fed intermittently, approximately 17 times in a day. Feeding precedes withdrawal. From Garber's description and diagrams of the digesters, it appears that the feed and withdrawal ports are sufficiently separated that direct bypassing of feed to the withdrawal point during a given feeding should not occur; however, since mixing occurs between feedings, the sludge withdrawn in the next feed-withdrawal cycle could be contaminated with feed from the previous cycle.

Samples of sludge for bacterial analyses were collected and cooled in a refrigerator before shipment. They were then shipped by air to Cincinnati in containers cooled with water ice. Time between sampling and the start of the bacterial analyses was approximately 24 hours. Samples for viral analyses were frozen in dry ice before shipment, transported in containers cooled with dry ice, and maintained at -70°C until extracted and assayed.

Procedures for virological determinations have been described by Berg and Berman.[8] Viruses were determined by the plaque technique on BGM cells. Membrane filter procedures were utilized for fecal coliforms (FC), total coliforms (TC), and fecal streptococci (FS), using standard procedures.[15] Salmonella sp. were assayed by the method of Kenner and Clark.[16]

Analyses for parasites were only carried out for Ascaris sp. by methods developed by investigators at the University of Cincinnati. Two methods were used. The first procedure gave numerous false positives and was modified about halfway through the investigation. The method and early results are not discussed. In the second, hypochlorite solution was used to eliminate much of the interfering

organic matter. These were far fewer false positives. This method has been reported by Meyer et al.[17]

Ohio Experiments

Digesters at Fairfield and Lebanon, Ohio, were sampled in this program. The Fairfield digester is actually three equally sized digesters in series. Operation is fill and draw, with feed charged to the first and withdrawn from the third digester. The Lebanon is a single-stage digester. Sludge is fed daily. Supernatant is also withdrawn daily and recirculated to the head end of the wastewater treatment plant. Sludge is withdrawn at the operator's convenience and discharged to the sand beds, weather permitting. During the winter, when drying rate slows down, sludge is withdrawn less frequently and the digester tends to become overloaded with solids. For both digesters, sampling did not coincide with feeding and drawdown. Feed was sampled by withdrawing a sample of sludge from the primary clarifier. Digested sludge was sampled directly from the digester (the third digester in series at Fairfield).

Bacterial procedures were the same as used in the Los Angeles tests, except the Salmonella sp. procedure was Kenner and Clark's[16] as modified by Venosa et al.[18]

RESULTS

Results are presented first for the Los Angeles experiments, which are compared at a later point with data on the Ohio digesters. The testing period covered approximately 2-1/4 years. During this time a total of 28 sample sets were taken for bacterial analyses, 12 sample sets for viral analyses, and about 16 sample sets for parasite determinations. Samples were taken from both the mesophilic digesters and the thermophilic digester. The sampling with respect to time was not completely uniform, but all seasons were covered over the course of the 2-1/4 year period. Table I presents average solids content, temperatures, and residence times during the testing period. Variation in temperature for the thermophilic digestion is greater than Garber indicates as desirable. Note, however, that this is not a daily variation but more nearly a weekly variation. Daily variation is much lower.[14] Presumably, a relatively large weekly variation is slow enough not to thermally shock sensitive thermophilic bacteria.

Bacteria

Mesophilic Conditions. Bacterial densities for fecal indicators and for Salmonella sp. are presented in Figures 1 and 2.

Examination of the curves at corresponding quarters of the years does not reveal any seasonable variation in density for any of the organisms.

TABLE I. Average Operating Conditions of the Los Angeles Anaerobic Digesters During 28-Test Sampling Program

| Conditions | Feed | | Product | | | |
|---|---|---|---|---|---|---|
| | | | Mesophilic | | Thermophilic | |
| | $\bar{x}$[1] | $s$[2] | $\bar{x}$ | $s$ | $\bar{x}$ | $s$ |
| Temperature (°C) | | | 35.6 | 0.51 | 49.1 | 1.17 |
| Sludge Solids Content (Wt.2%) | 5.70 | 1.26 | 2.52 | 0.34 | 2.21 | 0.34 |
| Nominal Residence Time (da) | | - | 20 | - | 20 | - |

1. $\bar{x}$ average of parameter
2. $s$ standard deviation of parameter

The densities of the fecal indicators in the incoming raw sludge were examined for correlation with Salmonella sp. densities. All three fecal indicators showed significant correlation coefficients ($r$):

| | $r^2$ | F - test |
|---|---|---|
| FC | 0.47 | F1/25 = 21.8 |
| TC | 0.55 | F1/19 = 23.4 |
| FS | 0.14 | F1/22 = 3.53 |

The correlations, although interesting, are of no particular value in predicting densities of salmonellae. For example, even with fecal coliform (FC), which showed the highest degree of correlation, the correlation explains less than half of the very large variation in Salmonella sp. densities (see Figure 2). The correlation was reduced but was still significant when the calculation was repeated without the raw sludge data from a questionable sludge sample set collected late in the first quarter of 1975 (see Figures 1 and 2--all four bacterial densities were very low for an unknown reason, but possibly because of the presence of a toxic waste in the feed). A high degree of correlation between a pathogen and the fecal indicators is not normally expected, because discharge of the pathogen represents disease incidence which normally rises and falls throughout the year, whereas the fecal indicators should not. However, a large geographically spread-out city such as Los Angeles may resemble the sum of many separate communities. Peaks and valleys in infection may be smoothed out so that commonly occurring pathogens like Salmonella sp. could take on the character of a fecal indicator and correlate with them.

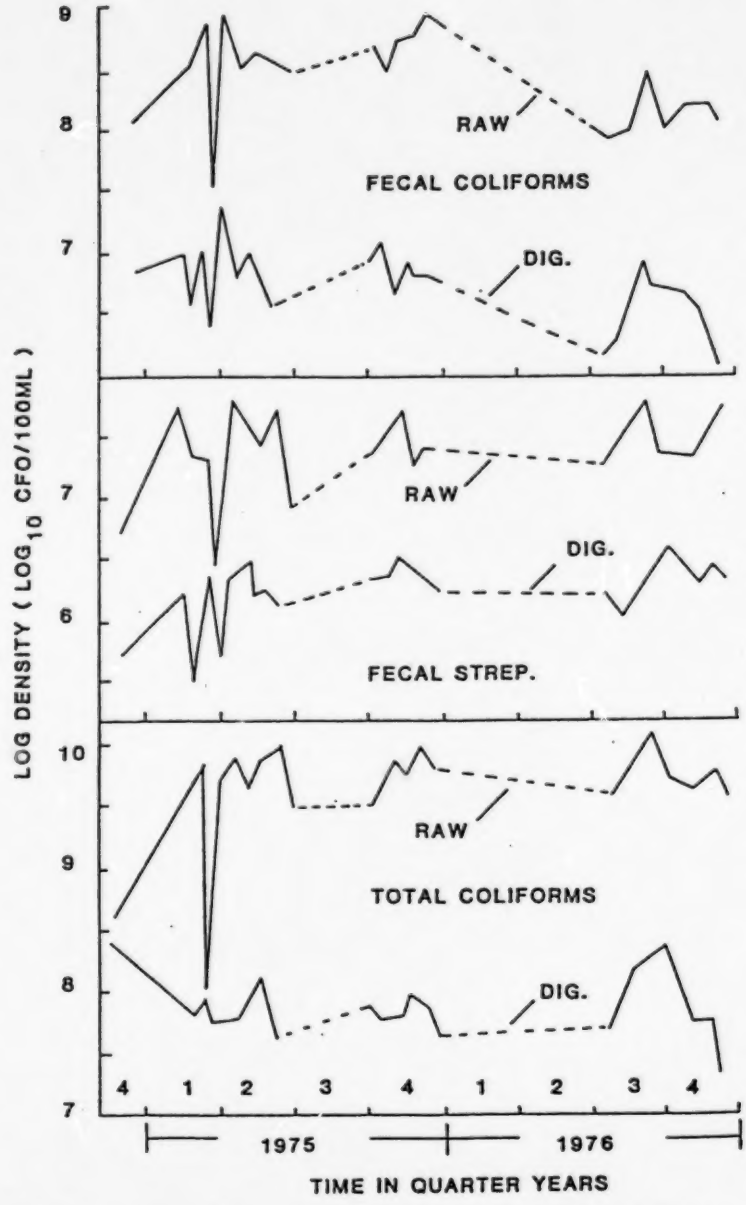


Figure 1. Fecal Indicator Densities of Raw and Mesophilically Digested Sludge, Los Angeles' Hyperion Plant.

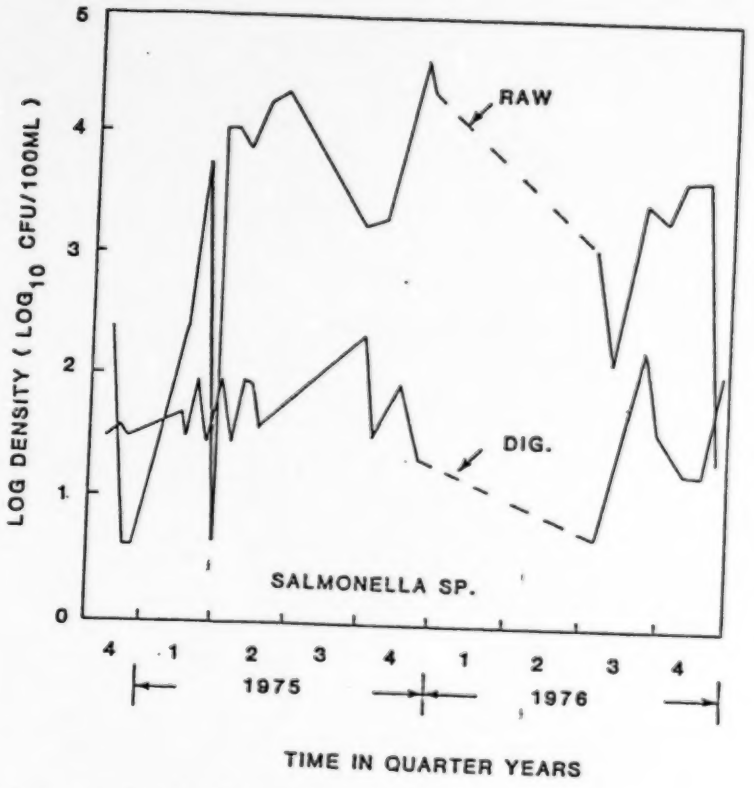


Figure 2. Salmonella Sp. Densities of Raw and Mesophilically Digested Sludge, Los Angeles' Hyperion Plant.

A primary objective of this investigation was to determine the effect of digestion on bacterial species, especially pathogens. Figures 1 and 2 compared bacterial densities in sludge before and after mesophilic anaerobic digestion. The data show, on the average, a uniform reduction in average log densities. Results are tabulated in Table II. Reductions in the average log density values and their 95% confidence limits are shown below:

| | | | |
|---|---|---|---|
| Sal | $1.45 \pm 0.25$ | FS | $1.12 \pm 0.09$ |
| FC | $1.73 \pm 0.09$ | TC | $1.78 \pm 0.11$ |

Fecal and total coliforms are evidently less resistant than salmonellae, whereas fecal streptococcus are more resistant and show smaller log reductions.

TABLE II. Salmonella and Fecal Indicator Densities in Raw and in Mesophilically and Thermophilically Digested Sludge (Densities are log CFU per 100 ml)

| Sludge Type | Salmonella (SAL) | Fecal Coliform (FC) | Fecal Streptococcus (FST) | Total Coliform (TC) |
|---|---|---|---|---|
| **Raw** | | | | |
| $\bar{x}$ | 3.09 | 8.43 | 7.36 | 9.63 |
| s | 1.23 | 0.38 | 0.34 | 0.46 |
| 95% CL of $\bar{x}$ | 2.6-3.6 | 8.3-8.6 | 7.2-7.5 | 9.4-9.8 |
| No.Measurements | 27 | 27 | 24 | 21 |
| **Mesophilically Digested** | | | | |
| $\bar{x}$ | 1.64 | 6.71 | 6.24 | 7.85 |
| s | 0.36 | 0.29 | 0.27 | 0.23 |
| 95% CL of $\bar{x}$ | 1.5-1.8 | 6.6-6.8 | 6.1-6.3 | 7.7-7.9 |
| No.Measurements | 27 | 26 | 24 | 21 |
| **Thermophilically Digested** | | | | |
| $\bar{x}$ | <0.48 | 3.93 | 4.22 | 4.47 |
| s | 0 | 0.82 | 0.72 | 0.98 |
| 95% CL of $\bar{x}$ | - | 3.6-4.2 | 3.9-4.5 | 4.0-4.8 |
| No.Measurements | 27 | 27 | 26 | 23 |
| **Reductions in $\bar{x}$** | | | | |
| Raw-Meso | 1.45 | 1.73 | 1.12 | 1.78 |
| Calcd.Std.Error | 0.25 | 0.093 | 0.089 | 0.11 |
| Calcd.95% CL | 0.96-1.94 | 1.54-1.92 | 0.94-1.30 | 1.56-2.00 |
| Raw-Thermo | >2.61 | 4.51 | 3.13 | 5.16 |

The data have a natural pairing, since samples on a given day were taken nearly simultaneously at inlet and outlet. However, a check of the data for correlation showed essentially no correlation between feed and product densities for Salmonella sp., fecal coliform, and total coliform. Correlation for fecal streptococcus was significant as indicated by the F-test ($r^2$ = 0.33, F1/20 = 9.97), although only 33 percent of the variation was explained by correlation. The lack of correlation indicates that there is little value in determining individual differences between feed and product sludge on a given day and averaging the differences (instead of determining the differences of the averages as was done above). Unless this procedure reduces standard deviation considerably, it is counterproductive because it results in a wider confidence interval for the average difference.

The density data are on the basis of 100 ml of sludge. To make the results comparable to other sites where solids concentrations entering and leaving are different, it is useful to examine data on the basis of mass of solids. These results are presented in Table III. The log reductions of the various organisms are substantially lower on the unit-weighted basis than on the 100 ml basis because solids content of digested sludge is lower than the incoming sludge (assuming no thickening step). The loss in solids content is high for Los Angeles sludge. Reasons are dilution by steam condensate, since the digesters are heated by direct steam injection (ca. 3% increase in volume), loss of grit, which deposits in the digester, and efficient digestion, which causes disappearance of volatile solids.

TABLE III. Log Reductions in Bacterial Densities on a Sludge Solids Basis

| | Salmonella | Fecal Coliform | Fecal Streptococcus | Total Coliform |
|---|---|---|---|---|
| Average Log Density in Sludge | | | | |
| Raw | 2.33 | 7.68 | 6.60 | 8.87 |
| Mesophilic | 1.24 | 6.31 | 5.84 | 7.45 |
| Thermophilic | <0.13 | 2.59 | 3.88 | 4.12 |
| Log Reduction | | | | |
| Raw - Meso | 1.09 | 1.37 | 0.76 | 1.43 |
| Raw - Thermo | >2.20 | 5.09 | 2.72 | 4.75 |

## Thermophilic Digestion

The thermophilic digester at Los Angeles was sampled and examined for the same organisms as the mesophilic digester. Density data are presented in Table II on the basis of 100 ml of sludge. Of the 27 measurements for Salmonella sp., all showed reduction to below detectable limits. Reductions in the average log densities were greater than 2.6. Considering the fact that salmonella were never detected in the digested sludge, the reduction is probably substantially greater than 2.6. The fecal indicators showed reductions in average log densities ranging from 3.1 to 5.2 with fecal streptococcus showing the least reduction.

Summarized results with densities based on a unit mass of solids are presented in Table III. As noted earlier for the mesophilic results, reductions in average log densities are substantially lower than on the basis of 100 ml of sludge but are still substantial.

## Viruses

Virus analyses were determined during three periods of the sampling program. These data are presented in Table IV, and are also presented graphically in Figure 3. Unlike the data for bacteria, the viral densities for the raw sludges show different densities for the different periods of the year. Berg et al.[8] suggested a seasonal effect. The dotted line in Figure 3 shows that a curve with an approximate sine wave shape, peaking in late summer with a minimum in the first quarter of the year, could explain the variation in density of the raw sludge. In Figure 3, the lines pointed out by arrows have been transposed from their actual time period to the preceding or following year to illustrate the fit of the sine-wave-shaped curve to the data.

TABLE IV. Effect of Anaerobic Digestion on Densities of Enteric Viruses in Sludge

| DATE | RAW SLUDGE | | MESOPHILIC | | THERMOPHILIC | |
|---|---|---|---|---|---|---|
| | L-PFU-V[1] | Percent Solids | L-PFU-V | Percent Solids | L-PFU-V | Percent Solids |
| 4/29/75 | 2.58 | 5.34 | 1.48 | 2.80 | <0.60 | 2.0 |
| 5/6/75 | 2.74 | 5.78 | 1.70 | 2.15 | <0.49 | 2.0 |
| 5/20/75 | 3.17[3] | 6.36 | -- | 1.83 | <0.45 | 2.0 |
| 6/3/75 | 2.97 | 5.8 | 1.60 | 1.9 | <0.43 | 1.4 |
| 10/21/75 | 3.44 | 4.5 | 2.45 | 2.4 | 0.52 | 2.0 |
| 11/4/75 | 3.19 | 4.4 | 2.40[3] | 3.0 | <0.15 | 2.4 |
| 12/2/75 | 3.08 | 6.2 | 2.38 | 2.7 | <0.23 | 2.1 |
| 12/16/75 | 3.26 | 6.0[2] | 2.00 | 2.7[2] | 0.23 | 2.1[2] |
| 8/17/76 | 4.06 | 7.36 | 2.61 | 2.7 | 0.69 | 1.6 |
| 8/31/76 | 3.63 | 6.3[2] | 2.67 | 2.7[2] | 0.76 | 2.0[2] |
| 9/14/76 | 3.39 | 5.3 | 2.30 | 3.0[2] | 0.66 | 2.8 |
| 9/28/76 | 3.19 | 3.5 | 2.56 | 2.7 | 1.22 | 2.4 |
| Ave. Temp. (°C) | | | | 35.6 | | 49.5 |

1. L-PFU-V = $LOG_{10}$ Plaque Forming Units/100 ml of sludge
2. Estimated values
3. These values differ slightly from the tabulation given by Berg et al.[8]

Log viral densities in mesophilically digested sludge as well as in the raw feed sludge are shown on Figure 3. There appears to be a constant log reduction caused by the digestion. To estimate the reduction in log densities caused by digestion, three approaches have been taken. In Method 1, the same procedure used for bacteria was followed; that is, average log densities of raw and digested sludge were determined and the difference and its confidence limits were calculated. Two other approaches were taken because of the

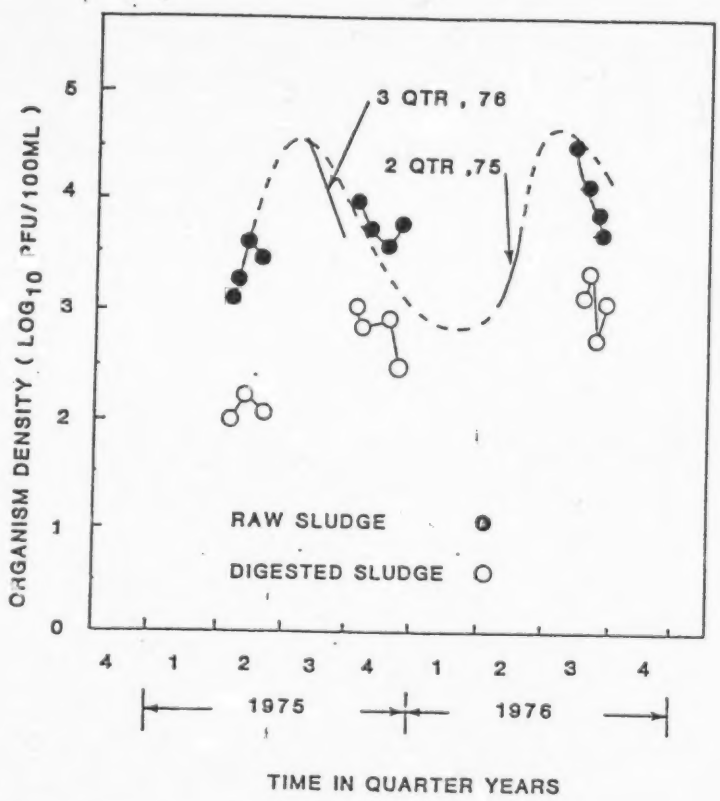


Figure 3. Virus Densities of Raw and Mesophilically Digested Sludge, Los Angeles' Hyperion Plant.

evident drift in densities of both raw and digested sludge with time. In Method 2, the averages of log densities for raw and digested sludge for each of the three blocks of data were determined, differences were calculated, and the three differences were averaged and confidence limits calculated. In the third method, log differences between the raw and digested sludge were calculated for each day sampled, the 11 differences were averaged and confidence limits of this difference were calculated. Results of the three approaches are shown below:

| Method | 1 | 2 | 3 |
|---|---|---|---|
| Ave.Log Difference (L-PFU-V)* | 1.03 | 1.08 | 1.03 |
| Standard Error | 0.17 | 0.10 | 0.078 |
| 95% Confidence Interval | 1.03 ± 0.35 | 1.08 ± 0.43 | 1.03 ± 0.17 |

* L-PFU-V = Log plaque-forming units per 100 ml

Determining the average of the eleven log differences (Method 3) produced the closest confidence limits.

As noted for bacteria, it is useful to consider the reduction in density on the basis of the mass of sludge solids rather than the volume of sludge. Log densities per gram of sludge solids can be calculated. This has been done by Method 3 and results are as follows:

| | |
|---|---|
| Ave. Log Difference (L-PFU-M)* | 0.71 |
| Standard Error | 0.060 |
| 95% Confidence Interval | 0.71 ± 0.13 |

*L-PFU-M = Log Plaque-Forming Units/gram of sludge solids

Thermophilic Digestion

The reductions in viruses caused by digestion at 49.6°C are substantially greater than the reductions at 35.6°C. Data show some survival. The log differences are shown on a per 100 ml and a per gram basis below:

| | log difference |
|---|---|
| L-PFU-V | > 2.61 |
| L-PFU-M | > 2.25 |

For the measurements that showed no viruses (about half of the total), the minimum detection limit was used in place of the unknown concentration. The average log reduction is thus greater than the value shown.

Parasites

Two sets of information were collected on parasite numbers and viability. The first set of data turned out to be essentially methods development. In the second set of six sample sets, intact Ascaris eggs were found in raw, mesophilic and thermophilic sludge. Densities were about the same (ca. 5-10 per 75 ml sludge) in all three sludges. In every sample, except for one mesophilic sludge sample, some evidence of viability was observed. Survival was expected for mesophilically digested sludge, but some destruction was expected for thermophilic digestion. The reliability of this result is open to question since samples were few, densities of the Ascaris sp. low, and analytical methods were in a state of continuous development.

Experiments with Other Digesters

Data on densities of bacterial indicators and Salmonella sp. have been routinely collected over the course of a year on influent and effluent from anaerobic digesters at Lebanon and Fairfield, Ohio. Details of operation are presented in Table V. Densities for fecal coliform and Salmonella sp. per 100 ml of sludge are presented on a logarithmic scale as a function of days since the start of the study in Figure 4 (for Fairfield) and Figure 5 (for Lebanon).

TABLE V. Average Operating Conditions for Fairfield and Lebanon Anaerobic Digesters

| | Fairfield | Lebanon |
|---|---|---|
| Temperature (°C) | 35 (No. 1)<br>32 (No. 2)<br>32 (No. 3) | 36 |
| Residence time (da) | 25 (design) | 30-60 (design) |
| Type of Sludge | Primary | Mixed Primary and Waste Activated |
| Nominal Wastewater Flow (MGD) | 5-7 | 1 |

For both cities, the reduction in fecal coliforms is substantial, averaging about 3.0 logs for Fairfield and 3.5 logs for Lebanon. Reductions in Salmonella sp. are difficult to quantify because influent levels are relatively high and many observations were below detectable limits (for Salmonella, detection limit was 3 CFU/100 ml). When sludge feed and product are both below detectable limits the data give no information. The data give minimal information when, as frequently occurred, the feed shows a low density and the product density is below the detection limit. For example, if the feed showed 6 CFU/100 ml and the product density was <3 CFU/ml, the log reduction would be greater than log 3/6 (-0.301), but it could be much greater than this. Examination of the Lebanon data for Day >220 indicates a log reduction greater than about 1 log--again, it is not certain how much greater. The period from 180 to 220 days shows unusually high Salmonella sp. densities in the product sludge. This may have been the result of a process upset or an overfeeding event, because the fecal indicators also peaked at that time.

For Fairfield, low Salmonella sp. densities in the feed complicate the interpretation of the data just as for Lebanon. Up to about Day 220, the occasional peaks in the feed indicate that reductions of between 1 and 2 logs occurred. The situation is reversed for two points below 250-260 days. Product sludge showed higher densities than the feed. No explanation of this anomalous behavior is offered. The uusal straw to grasp at is to attribute to anomaly to an "unusual unreported operating procedure," which may or may not be the case.

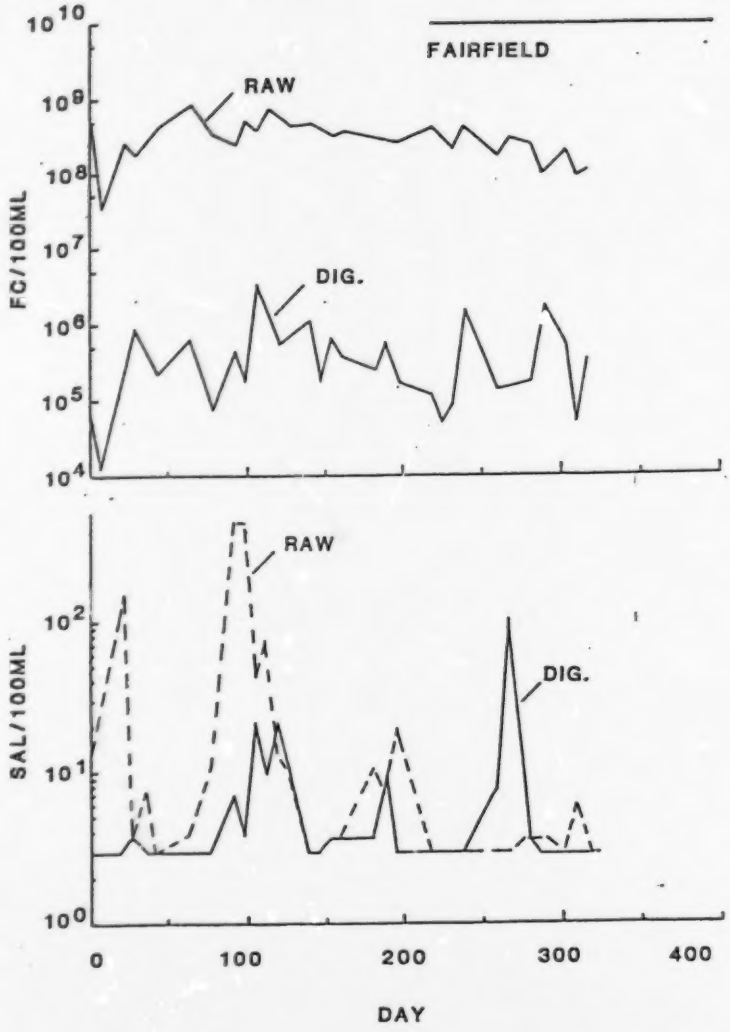


Figure 4. Fecal Indicator and Salmonella Sp. Densities of Raw and Mesophilically Digested Sludge, Fairfield, Ohio

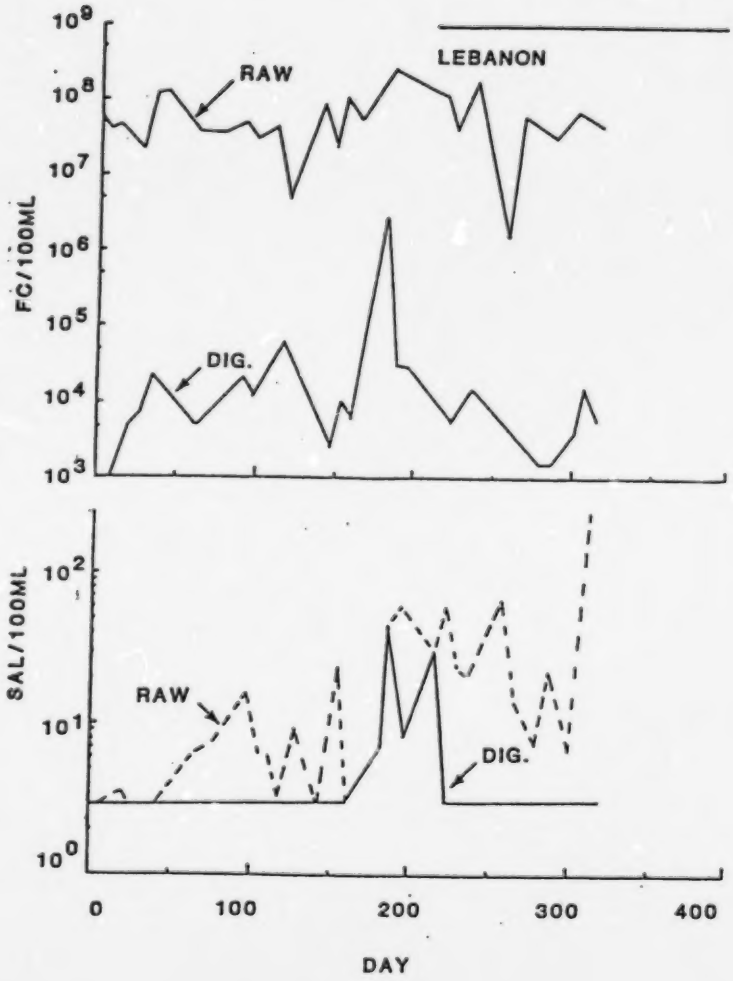


Figure 5. Fecal Indicator and Salmonella Sp. Densities of Raw and Digested Sludge, Lebanon, Ohio.

## DISCUSSION

Experimentation with full-scale continuous flow wastewater treatment processes presents special problems compared to other forms of industrial research for the following reasons:

1. Feedstock is extremely variable in composition.
2. Residence times are long. For sludge digestion, residence times are in weeks rather than the fractions of an hour typical of, for example, chemical processing.
3. For microbiological parameters, compositing of samples to smooth out fluctuations is of limited value.

The usual answer to this problem is to analyze large numbers of input and output samples over a total length of time substantially exceeding process residence time while at the same time keeping process conditions constant. If input parameters show no long-term trends, this approach should be adequate. This has been true for our estimates of the reductions in bacterial densities that occur in mesophilic digestion from the Los Angeles data. Plotting the data against time (Fig. 1 and 2) established that time trends did not exist, and confidence limits could be established for the relative reductions caused by mesophilic digestion. For thermophilic digestion, reductions could be estimated well for bacterial indicators but not for Salmonella sp. The cause of the difficulty was not the experimental approach but the low densities of salmonellae in the influent; densities were not sufficiently high that there were any survivors in the digested sludge.

The problem of lack of sufficient density of Salmonella sp. in the influent sludge significantly reduced the value of the information collected at the Lebanon and Fairfield plants. The data cannot be used to corroborate the information obtained from the Los Angeles data on the reduction of Salmonella sp. densities relative to the various fecal indicators. Either the sensitivity of the Salmonella sp. determination must be increased or plants selected with a high influent density of salmonella.

The unexplained excursions in Salmonella sp. densities in the digested sludge at Fairfield and Lebanon arouse the suspicion (possibly unwarranted) that unusual operating conditions caused the excursions. It is important to be sure that the plant operation is stable during the testing period, which probably is best accomplished by having an active and informed cooperator at the test site.

Interpretation of virological data obtained at Los Angeles required a different approach than the bacteriological data because of the drift with time of the incoming virus densities in the raw sludge. Pairing of raw and digested sludge samples collected on a given day eliminated the effect of this trend and reduced variability. Fortunately, viral densities in the incoming sludge were sufficiently high that densities in the outgoing sludge could still be measured. Insufficiently high incoming viral densities interfered with interpretation of results in the extensive investiga-

tion of viral reductions in digestion conducted by Lydholm and Nielson.[19] If attempts at verifying the virus reductions at Los Angeles are carried out at other locations, pretesting should be conducted to determine whether incoming virus levels are adequate to permit quantitative statements to be made about extent of reductions.

The pairing of results obtained on same-day samples offers no advantages for reducing variability except for eliminating the effects of long-term trends unless there is direct bypassing from inlet to outlet. Such bypassing is possible if charging precedes withdrawal, mixing continues during the operation (mixing by gas evolution occurs at all times, especially after feeding), and if both operations take more than several minutes to accomplish. In this case, pairing of an input sample and a sample of the sludge as it is withdrawn will reduce variability (unfortunately, such direct bypassing degrades pathogen reduction). Tracer tests can also be used to find when a digester withdrawal stream is most influenced by a given digester charge. Because microbial species densities are reduced by the adverse conditions in the digester, the optimum time for observing an effect of feed on the microbial densities in the stream withdrawn would be less than the time indicated by a tracer test. If sludge is charged and withdrawn once a day and the digester is reasonably well mixed, pairing of the sludge fed one day with the sludge withdrawn the next day will substantially reduce variability. Such an approach will not bias the calculated average reduction in log density.

The performance of the Fairfield digester in reducing fecal coliform and Salmonella sp. densities appeared approximately equivalent to the Lebanon digester. This is an unexpected result, considering that the multistage operation of the Fairfield digester would greatly reduce contamination of the sludge being withdrawn with sludge with a relatively short residence time in the digester. The result indicates that other factors besides temperature and actual residence time of the sludge determine bacterial survival.

The investigation presents no proof that digestion should reduce bacterial or viral densities by constant factors, or, equivalently, by constant log reductions. However, an analysis of the rate equations for a completely mixed vessel indicate that correlation of the log reductions with other variables is reasonable. If rate constant for decline of microbial density is approximately first order (which is expected), digestion is completely mixed, and reductions in density of about 10-fold are experienced, analysis of the rate equations indicate that ratio of effluent to influent density is independent of absolute influent density and is approximately inversely proportional to residence time. If residence time should be increased over the range normally encountered in digestion (15 to 30 days), log reduction is predicted to increase by 0.3 log (i.e., a factor of 2). Log reductions then should be relatively insensitive to influent densities and small differences in residence time.

## CONCLUSIONS AND RECOMMENDATIONS

Data obtained on Los Angeles sludge defines the reduction in certain bacteria and viruses by mesophilic anaerobic digestion and allows definition of defensible confidence limits. Attempts to supplement these data with information on bacterial reductions produced by other digesters were handicapped by insufficiently high densities of Salmonella sp. in the influent which resulted in below detectable densities in the effluent. Further studies should be conducted either with improved measuring methods, or plants should be selected which have demonstrated consistently high densities of salmonellae in the influent.

A multistage digester showed bacterial reductions equivalent to a single stage digester under approximately equivalent residence time. This unusual result should be investigated further, preferably on a closely controlled pilot scale.

Reductions in Ascaris sp. by thermophilic digestion at 49°C were poor, although data were preliminary in nature. More work should be done to establish the temperature levels needed in thermophilic digestion (anaerobic and aerobic) to destroy Ascaris eggs.

## ACKNOWLEDGEMENT

The authors are members of EPA's Water Engineering Research Laboratory in Cincinnati. Thanks are due to Messrs. Nagano, Ohara, and Garber at Los Angeles' Hyperion Wastewater Treatment plant who contributed greatly to this investigation.

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